

Influence of fining agents on the sensorial characteristics and volatile composition of mead

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Mead, one of the oldest fermented drinks, is derived from the fermentation of diluted honey by yeasts. In the context of wine production, several procedures are applied to stabilize the beverage and to improve its organoleptic properties. This study aims to evaluate the impact of adding fining agents on the production of mead. In general, the best results were obtained for the samples containing just one fining agent instead of two combined. However, the best performance was obtained for the combined fining agents (bentonite + gelatine + egg albumin). Tannins decreased significantly the content of volatile compounds. On the other hand, silica appears to be the best fining agent, resulting in the lowest loss of volatile compounds. Thirty-six volatile compounds were determined by gas chromatograph–flame ionization detector and gas chromatography–mass spectrometry, including alcohols (42.5%), carbonyl compounds (40.4%), acetates (14.4%) and esters (1.8%). Eleven volatile compounds had odour activity values >1, representing those with a major impact on the aroma of mead. Significant differences ($p < 0.05$) were found in 10 volatile compounds independently to the type of treatment used and no differences ($p > 0.05$) were observed for remaining compounds. Copyright © 2017 The Institute of Brewing & Distilling

Keywords: mead; fining agents; sensorial analysis; volatile compounds; odour activity values

Introduction

Mead is a traditional alcoholic drink derived from the fermentation of diluted honey performed by yeasts and is, perhaps, the oldest fermented drink in the world (1,2). In general, the mead-making process comprises several steps, including must preparation and pH adjustment followed by must pasteurization, yeast inoculation, fermentation and post-fermentation. Finally, the mead is centrifuged in order to remove undesired material (2).

During the mead-making process, as happens for wine, the presence of foreign matter in the liquid is common. In wine production, turbidity is visible owing to the presence of foreign matter from various sources, including cellular debris, grape pulp, ground particles, insoluble waste products of wine treatments and microorganisms (3). In the particular case of mead some particles may be observed that are present in honey, like pollen grains, but also residues from various other sources. On the other hand, after fermentation, visible turbidity results from the accumulation of dead yeast cells or even remnants of certain additives, like nutrients that are added during the fermentation process as adjuvants. Therefore, it is extremely important to develop procedures that provide stability to the final product and improve its organoleptic characteristics, particularly clarity, which is a major demand of consumers. Thus, the fining of the final product aims to ensure taste quality, undisturbed by undesired precipitates, also allowing the removal of colloids and particulate matter that can be removed by decantation, filtration or centrifugation. Therefore, the fining process in alcoholic beverages plays an important role in oenology practices, ensuring wine's stabilization as well as providing or improving its organoleptic properties (4).

Fining agents are grouped according to their general nature as earths (montmorillonite, bentonite and kaolinite), animal protein (gelatine, isinglass and casein), plant protein (wheat gluten, soya and lupin), wood charcoal (carbons), synthetic polymers, silicon dioxide (kieselsohl), metal chelators and enzymes (pectinases) (5). Worldwide, the most used fining agents are sodium bentonite and proteins associated with tannins or a mineral agent (3).

Fining in the context of wine-making is an operation that consists of the addition of substances that are able to flocculate and settle, thereby removing particles that are in suspension (6).

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The fining of the final product should be permanent, meaning that the method ensures the stability of the product for a long time (7,8).

It is important to note that fining is not a standard process since the action of fining agents depends on the substances with which they interact. There are several types of fining from different sources that interact with diverse components differently (7,8). In addition to removing insoluble material, fining has been reported to be responsible for elimination of organic acids, nitrogen compounds and some phenolics of colloidal nature that are implicated in oxidation and excess astringency and bitterness. Thus, apart from improving the physical and chemical stability of the final product, fining agents enhance organoleptic characteristics (3,4,6,8).

There are two major sub-groups of fining technique depending on the equipment applied: *dynamic*, using centrifuges, filters and floats; and *static* that associates fining agents with refrigeration, sulphite and enzymatic treatments (9).

According to the literature, fining agents also act as insoluble solids promoting yeast growth and allowing a faster and more complete fermentation (10). According to Úbeda (11), the mechanism is relatively simple and relies on attraction of charged particles of opposite charge; this attraction (or clotting) cancels charges and causes the formation of flocs, which will increase in size and weight and may drag other substances down during precipitation.

In the present study, static finings were used, also known as 'glues', with the aim of accelerating the spontaneous fining that occurs mainly after fermentation. Therefore this study aims to evaluate the influence of commercial fining agents on mead production.

Materials and methods

Samples

In this study, dark honey for mead production purchased from a local beekeeper in the northeast region of Portugal (Bragança) was used. Physicochemical (moisture content, ash content, free acidity, reducing sugars and apparent sucrose, hydroxymethylfurfural, diastase activity and water activity) and microbiological (aerobic mesophiles, moulds and yeasts, faecal coliforms, sulphite-reducing clostridia and salmonella) analyses of the honey were performed (results not shown).

Preparation of honey must for fermentation

In order to obtain an alcoholic beverage with an alcoholic strength (AS) by volume of ~11%, honey was diluted in natural spring water obtained from the market (370 g/L), and mixed to homogeneity as previously described (12).

The chemical composition of the water used was: pH (at 18°C), 6.32; HCO₃⁻, 21.7 mg/L; Cl⁻, 8.3 mg/L; Na⁺, 9.3 mg/L; Ca²⁺, 6.2 mg/L; SiO₂, 16.0 mg/L; dry residue at 180°C, 70.0 mg/L; total mineralization, 80.0 mg/L, according to the label information on the container.

For mead production, steps described previously (2) were followed with slight modification in the final product. Before fermentation, various physicochemical parameters such as total soluble solids (°Brix), pH, total acidity (TA), expressed as tartaric acid, yeast assimilable nitrogen (YAN) and reducing sugars (RS)

concentration were determined according to standard methods (13). The pH was adjusted to 3.5 with tartaric acid (Sigma Aldrich, Italy). The honey musts were pasteurized at 65°C for 10 min and immediately cooled at 4°C.

Yeast inoculation

The starter cultures were prepared by hydration of 0.3 g of active dry yeast *Saccharomyces cerevisiae* Lalvin ICV D47 (Lallemand, Montreal, Canada) in 3 mL of honey must and maintained in a water bath for 15 min at 35°C (12,14).

Control of fermentation conditions and monitoring

The fermentations were carried out according to the literature (15). The honey must, previously pasteurized and cooled, was inoculated with activated yeasts of 10⁵ colony-forming units (CFU/mL). Then, the flasks with inoculated honey must were incubated at 25°C with shaking (120 rpm/min). Fermentations were monitored daily by measurement of the optical density at 640 nm and reducing sugars (RS) at 540 nm using a Unicam Helios Alpha UV-visible spectrometer (Thermo Spectronic, Cambridge, UK), respectively. For RS determinations, the 3,5-dinitrosalicylic acid method was performed using glucose as the standard. At the end of alcoholic fermentation, some physicochemical parameters such as pH, TA, volatile acidity (VA), expressed as acetic acid, YAN, total SO₂, AS and final RS concentration were determined according to the standard methods (13). YAN was determined by the formaldehyde method as described elsewhere (12).

Fining procedures

In order to identify the best fining agents, as well as the most suitable concentration to be used for mead clarification, preliminary tests were carried out with different concentrations (low, medium and high) following the instructions of the supplier. After addition of the fining agent, mead remained undisturbed for 4–7 days in a dry, cool environment. After the fining process, samples were taken for further study of their volatile composition. Various finings agents such as casein, gelatine, silica, bentonite, egg albumin and tannins and combined fining agents (bentonite + gelatine + egg albumin), purchased from AEB Group Company (Bioquímica Portuguesa SA), were subjected to preliminary laboratory tests (Table 1). The following concentration of fining agents was tested: B (bentonite, gelatine and egg albumin) = 1.5 g/L; S (silica) = 1 mL/L; T (tannins) = 0.3 g/L; G (gelatine) = 1.0 mL/L; BS (bentonite, gelatine and egg albumin + silica) = B (1.5 g/L) + S (1.0 mL/L); TS (tannins + silica) = T (0.3 g/L) + S (1.0 mL/L); and SG (silica + gelatine) = S (1.0 mL/L) + G (1.0 mL/L).

It is important to highlight that the control/sample was not considered owing to the fact that our objective was to find the best fining agents for mead clarification and their influence on the volatile compounds composition.

Analysis of major volatile compounds

The major volatile compounds were directly analysed after adding 350 µg of 4-nonanol (internal standard) to 5 mL of each mead sample. A Chrompack CP-9000 gas chromatograph equipped with a split/splitless injector, a flame ionization detector (FID) and a Meta-Wax capillary column (30 m × 0.25 mm; 0.2 mm film thickness, Teknokroma) was used. The temperatures of the injector

Table 1. Commercial *fining agents* subjected to preliminary studies in our laboratory

| Code samples/ fining agents | | Commercial name (AEB) | Source | Form | Composition | Dosages | |
|--------------------------------|--|--------------------------|--|--------|--|-------------------|---------|
| | | | | | | Range | Highest |
| B | Bentonite + gelatine + egg albumin | Bioquickgel® | Very fine clay made of aluminium silicate + animal protein + the major protein found in egg whites | Powder | E558 bentonite + E372 alimentary gelatine + egg albumin | 50–150 (g/hL) | 150* |
| S | Silica | Spindasol®W | component of sand, glass and quartz | Liquid | Silica sol in aqueous 30% | 50–100 (ml/hL) | 100* |
| T | Tannins | Gallovín | Insect galls on oak leaves | Powder | Enological tannins 100% | 5–30 (g/hL) | 30* |
| G | Gelatine | Gelsol® | Animal protein | Liquid | Alimentary gelatine 50%; E228 Potassium bisulphate, etc. | 5–100 (ml/hL) | 100* |
| C | Casein | Micron® 96 | Principal protein in milk | Powder | Micronized potassium caseinate | 20–100 (g/hL) | 100 |
| Bt | E558 bentonite | Bentogran® | Very fine clay made of aluminium silicate | Powder | Aluminium silicate hydrated | 10–40 (g/hL) | 40 |
| Eg | Egg albumin | Ovogel® | The major protein found in egg whites | Powder | Egg albumin | 5–10 (g/hL) | 10 |

* Selected fining agents and dosages used in this study.

and the detector were both set to 250°C. The oven temperature was initially held at 50°C, for 2 min, then programmed to rise from 50 to 177.5°C, at 5°C/min, then from 177.5 to 230°C at 10°C/min and finally maintained at 230°C for 15 min. The carrier gas was helium GHE4x (Praxair) at an initial flow rate of 1.0 mL/min (75 kPa at the head of the column). The analyses were performed by injecting 1.0 mL of sample in the split mode (15 mL/min). The quantification of major volatile compounds, after the determination of the detector response factor for each analyte, was performed with the software Star-Chromatography Workstation version 6.41 (Varian) modified by confirmation of several compounds with standard tests described in several previous works (12,16–21).

Extraction and analysis of minor volatile compounds

To a 10 mL culture tube (Pyrex, ref. 1636/26MP), 8 mL of mead sample, 3.5 µg of internal standard (4-nonanol) and a magnetic stir bar (22.2 × 4.8 mm) were added. Extraction was carried out by stirring the sample with 400 µL of dichloromethane according to Oliveira *et al.* (16). After cooling at 0°C during 15 min, the magnetic stir bar was removed and the organic phase was separated by centrifugation (4000 rpm, 7 min, 4°C) and the extract recovered into a vial using a Pasteur pipette. Then, the extract was dried with anhydrous sodium sulphate and placed into a new vial. Extractions of volatiles from each sample were carried out in duplicate. Volatile compound analysis was performed using a GC–MS constituting a Varian Saturn 2000 chromatograph with a 1079 injector and an ion-trap mass spectrometer. Samples of 1 µL were injected in splitless mode (30 s) into a Sapiens-Wax MS Teknocrroma column (30 m × 0.15 mm; 0.15 µm film thickness).

The temperature of the injector was held at 250°C. The temperature of the oven was held at 60°C, for 2 min, then programmed to rise to 234°C at 3°C/min, increasing from 234 to 260°C at 5°C/min and finally held for 5 min at 260°C. The carrier gas was helium GHE4x (Praxair) at a constant flow rate of 1.3 mL/min. The detector

was set to electronic impact mode (70 eV) with an acquisition range from 35 to 300 *m/z*. The identification of volatile compounds was performed using the software Star – Chromatography Workstation version 6.9.3 (Varian) modified by confirmation of several compounds with standard compounds performed in several previous works (12,16–21). Volatile compounds were determined, semi-quantitatively, as 4-nonanol equivalents.

Determination of odour activity values

Odour activity values (OAVs) were determined in order to evaluate the contribution of a specific chemical compound to the aroma of the mead under study. These measure of the specific contribution of a compound to the odour of a sample was calculated as the ratio between the concentration of an individual compound and the perception threshold (17,22). Only compounds with OAVs > 1 were considered to provide a significant contribution to the mead's aroma (21,23).

Statistical analysis

Each experiment was performed in duplicate and the results were expressed as mean ± standard deviation. Sensorial analysis was performed using XLSTAT 2015.1.01 program in order to determine the mead that would be preferred by consumers. For volatile compounds, analysis of variance (ANOVA) was performed using the general linear model procedure as implemented in SPSS software, version 20.0 (SPSS Inc.). All dependent variables were analysed using a one-way ANOVA. The post-hoc means tests were performed using Tukey's test. All statistical tests were performed at a 5% significance level.

Sensory analysis

The sensory evaluation of meads was performed according to procedures described by Ferreira *et al.* (24). After clarification, a partly

trained consumer panel (usual consumers of mead and similar products) carried out the sensory analysis in order to assess product's acceptance. The panel included 43 consumers – 32 men and 11 women, aged between 18 and 68 years old.

For each taster a plastic glass, properly coded, was used for tasting. Mineral water and unsalted crackers were used for cleansing the palate (24). The consumers were asked to evaluate separately each of the three samples on a hedonic scale of 1–5, with the values 5 = liked extremely; 4 = liked moderately; 3 = not liked and not disliked; 2 = disliked moderately; 1 = disliked extremely. The evaluated qualities attributes were flavour, aroma, sweetness, alcoholic content and general assessment. The scores were used to evaluate the overall quality of the mead under study.

Results and discussion

Composition of honey must and mead

The results of physicochemical analysis of honey must under study are displayed in Table 2. The final pH (3.4), RS (31.2 g/L) and YAN (46.7 mg/L) were lower than the value determined initially for the honey must. For other parameters such as TA, VA, SO₂ and AS, the final values were 5.1 g/L, 1.5 g/L, 32.4 mg/L and 11.5%, respectively. These results were relatively higher than those observed by Pereira *et al.* (21), except for pH and TA, which exhibited lower values in mead fermented by the same strain of *Saccharomyces* as used in this study. According to Pereira *et al.* (21), the discrepancies among these results may be explained by differences in medium composition and fermentation conditions. Although the yeast strain used was the same, the honey used for must preparation was different.

Effects of fining agents in the volatile composition of mead

In Table 3 can be observed the mean values and standard deviations (SD) regarding the concentration of volatile compounds identified in the mead samples resulting from the fermentation process carried out using *S. cerevisiae* Lalvin ICV D47. The compounds that exhibited higher concentrations, in decreasing order, were acetaldehyde, 3-methyl-1-butanol, ethyl acetate, 2-phenylethanol, 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, monoethyl succinate, acetoin, diethyl succinate, ethyl lactate, octanoic acid, 5-hydroxymethylfurfural, hexanoic acid, 2-

methylpropanoic acid and *trans*-furan linalool oxide. The remaining compounds exhibited concentrations <1 mg/L. It is important to highlight that, though some volatile compounds exhibited concentrations <1 mg/L (trace), this does not mean they do not contribute to the aroma of the beverage. In Fig. 1 can be seen the prevalence/percentages of the main groups of volatile compounds on mead under study.

Alcohols

The results show that the group of alcohols was the most prevalent of all volatile compounds identified in the mead samples under study, at 42.5%. This group was represented by 3-methyl-1-butanol with a concentration ranging from 94.0 to 137.1 mg/L, followed by 2-phenylethanol, 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, tyrosol, 3-ethoxy-1-propanol and 1-hexanol. Therefore, these findings are in agreement with those previously reported (25,26). Indeed, these authors found that 3-methyl-1-butanol is present in wine in the highest relative concentration, followed by 2-phenylethanol as second greatest relative amount. Also Pereira *et al.* (21) found this group to be the majority of all volatile compounds quantified, represented by 3-methyl-1-butanol with concentration above its perception threshold. Therefore, 3-methyl-1-butanol exhibited the highest concentration in all meads studied by these researchers. 2-Phenylethanol was the second most representative compound in the alcohol group. This compound has been reported to be one of compounds released from the metabolism of yeast (27). These compounds are synthesized by yeast via an anabolic pathway from glucose or a catabolic pathway of their corresponding amino acid (27). Phenylethanol appears which is characterized by appearing at the beginning of the fermentation, reaching a constant value during the fermentation and subsequently tending to decrease at the end of fermentation (28).

For this group, 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 2-phenylethanol and tyrosol were the compounds that exhibited significant differences for $p < 0.05$ only in the samples B (bentonite + gelatine + egg albumin) and TS (tannins + silica), while 1-hexanol and 3-ethoxy-1-propanol showed no significant differences at $p > 0.05$ in all samples under study.

Carbonyl compounds

The second group included carbonyl compounds (40.4%), represented by acetaldehyde, which presented higher concentrations in the sample S and the lowest in the sample B. Other compounds were also found in the following order of abundance: acetoin, 5-hydroxymethylfurfural, furfural and benzaldehyde. Studies done by Ebeler and Spaulding (29) reported that acetaldehyde is generally the aldehyde present in highest concentrations in sherry and aged wines. According to Longo *et al.* (30), their concentration in wine is 13–30 mg/L while Delteil and Jarry (31), using *S. cerevisiae* strain ICV D47, reported a concentration of 105 mg/L. Significant differences ($p < 0.05$) were only observed in acetaldehyde for samples B, S and TS. For the remaining samples, no significant differences ($p > 0.05$) were observed.

Acetates

Regarding the third group of acetates, which correspond, on average, to 14.4% of the volatile compounds available in mead

Table 2. Physicochemical analysis of honey must and meads obtained previously and after fermentation process

| Physicochemical Parameters | Honey must | Mead |
|--|--------------|------------|
| Total soluble solids (°Brix) | 24.0 ± 0.1 | n.d. |
| pH | 4.0 ± 0.0 | 3.4 ± 0.0 |
| Reducing sugars, RS (g/L) | 215.7 ± 0.0 | 31.2 ± 5.1 |
| Total acidity, TA (g/L) | 1.9 ± 0.1 | 5.1 ± 0.2 |
| Yeast assimilable nitrogen, YAN (mg/L) | 130.7 ± 10.7 | 46.7 ± 4.0 |
| Volatile acidity, VA (g/L) | n.d. | 1.5 ± 0.6 |
| SO ₂ , total (mg/L) | n.d. | 32.4 ± 2.8 |
| Alcoholic strength by volume, AS (%) | n.d. | 11.5 ± 0.1 |
| n.d., Not determined. | | |

Table 3. Retention time, mean concentration ([]) and standard deviation (SD) of major and minor volatile compounds analysed by GC-FID and GC-MS, respectively, in the mead treated by different fining agents

| Retention time | Identified volatile compounds (µg/L) | Mead (one fining agent) | | | | Mead (two fining agents) | | | |
|-----------------------|--------------------------------------|---------------------------------|------------------------------------|------------------------------------|------------------------------------|----------------------------------|----------------------------------|--------|--------|
| | | B | T | S | TS | B | TS | SG | BS |
| | | □ ± SD | □ ± SD | □ ± SD | □ ± SD | □ ± SD | □ ± SD | □ ± SD | □ ± SD |
| Alcohols | | | | | | | | | |
| 5.69 | 1-Propanol* | 40,723.0 ± 1081.4 ^b | 25,423.0 ± 6993.8 ^{ab} | 35,335.0 ± 2763.7 ^{ab} | 17,519.0 ± 1736.4 ^a | 37,685.0 ± 1663.0 ^{ab} | 29,012.0 ± 971.7 ^{ab} | | |
| 6.54 | 2-Methyl-1-propanol* | 23,028.0 ± 215.0 ^b | 15,341.0 ± 1336.8 ^{ab} | 17,598.0 ± 608.1 ^{ab} | 10,779.0 ± 1303.9 ^a | 20,670.0 ± 1522.6 ^{ab} | 14,957.0 ± 2638.0 ^{ab} | | |
| 9.09 | 2-Methyl-1-butanol* | 18,152.0 ± 1217.3 | 15,054.0 ± 1693.3 | 17,914.0 ± 2354.6 | 11,254.0 ± 683.2 | 16,545.0 ± 1263.4 | 14,007.0 ± 107.0 | | |
| 9.23 | 3-Methyl-1-butanol* | 137,143.0 ± 5738.3 ^c | 101,719.0 ± 10,161.1 ^{ab} | 118,086.0 ± 19,562.2 ^{ab} | 93,945.0 ± 7733.0 ^a | 119,531.0 ± 9853.0 ^{ab} | 107,913.0 ± 1983.6 ^{ab} | | |
| 10.46 | 1-Hexanol | 5.2 ± 0.9 | 3.0 ± 0.2 | 25.3 ± 1.6 | 4.0 ± 1.0 | 4.7 ± 0.7 | 5.7 ± 0.9 | | |
| 10.61 | 3-Ethoxy-1-propanol | 25.2 ± 2.5 | 15.8 ± 3.9 | 133.9 ± 7.8 | 15.5 ± 3.5 | 22.4 ± 3.9 | 39.0 ± 0.1 | | |
| 26.46 | 2-Phenylethanol* | 49,568.0 ± 7580.0 ^b | 30,813.0 ± 5388.8 ^{ab} | 32,935.0 ± 2845.5 ^{ab} | 19,551.0 ± 2676.4 ^a | 42,161.0 ± 1403.4 ^{ab} | 34,416.0 ± 4566.9 ^{ab} | | |
| 60.15 | Tyrosol | 139.4 ± 15.5 ^{ab} | 65.9 ± 7.9 ^a | 302.1 ± 8.5 ^b | 40.9 ± 2.2 ^a | 98.9 ± 7.2 ^{ab} | 111.7 ± 6.9 ^{ab} | | |
| | Subtotal | 268,783.8 | 188,434.7 | 222,329.3 | 153,108.4 | 236,717.9 | 200,461.4 | | |
| Carbonyl compounds | | | | | | | | | |
| 2.79 | Acetaldehyde* | 146,937.0 ± 2568.2 ^a | 160,586.0 ± 18,116.3 ^{ab} | 308,658.0 ± 59,358.1 ^c | 261,164.0 ± 29,217.1 ^{bc} | 161,530.0 ± 213.2 ^{ab} | 184,613.0 ± 4971.3 ^{ab} | | |
| 8.32 | Acetoin | 2071.8 ± 5.7 | 957.4 ± 278.7 | 12,594.6 ± 248.8 | 727.9 ± 83.5 | 2027.2 ± 982.7 | 3176.2 ± 60.9 | | |
| 14.33 | Furfural | 217.3 ± 4.1 | 146.7 ± 28.2 | 823.1 ± 45.8 | 158.0 ± 2.9 | 180.4 ± 35.3 | 259.5 ± 12.1 | | |
| 16.46 | Benzaldehyde | 33.6 ± 0.9 | 53.8 ± 0.9 | 123.2 ± 6.8 | 68.0 ± 6.1 | 29.7 ± 2.4 | 62.5 ± 8.9 | | |
| 47.72 | 5-Hydroxymethylfurfural | 605.8 ± 21.7 | 426.9 ± 70.7 | 1632.7 ± 224.7 | 404.3 ± 16.1 | 602.3 ± 55.1 | 973.8 ± 57.4 | | |
| | Subtotal | 149,865.5 | 162,170.8 | 323,831.6 | 262,522.2 | 164,369.6 | 189,085.0 | | |
| 34.58 | Diethyl malate | 200.0 ± 2.2 | 116.0 ± 21.6 | 459.0 ± 19.2 | 142.8 ± 25.2 | 185.2 ± 59.6 | 231.4 ± 6.8 | | |
| 44.65 | Monoethyl succinate | 5340.2 ± 117.4 ^{ab} | 3398.7 ± 620.8 ^{ab} | 15,352.1 ± 834.7 ^b | 356.0 ± 3.2 ^a | 5453.9 ± 608.5 ^{ab} | 7122.2 ± 453.2 ^{ab} | | |
| | Subtotal | 8887.7 | 5322.4 | 23,608.2 | 3022.9 | 8419.7 | 10,325.6 | | |
| Acetates | | | | | | | | | |
| 3.46 | Ethyl acetate* | 52,325.0 ± 1352.5 ^a | 60,602.0 ± 3841.0 ^{ab} | 114,406.0 ± 4215.0 ^d | 90,409.0 ± 6301.2 ^c | 57,773.0 ± 1547.2 ^{ab} | 68,130.0 ± 2265.4 ^b | | |
| 4.57 | Isoamyl acetate | 59.2 ± 0.4 | 63.2 ± 3.0 | 360.4 ± 5.8 | 101.7 ± 3.8 | 51.1 ± 6.8 | 81.7 ± 3.3 | | |
| 26.85 | 2-Phenylethyl acetate | 31.0 ± 0.4 | 33.8 ± 2.1 | 123.5 ± 6.7 | 45.5 ± 2.3 | 48.3 ± 4.8 | 54.3 ± 1.4 | | |
| | Subtotal | 52,415.2 | 60,699.0 | 114,889.9 | 90,556.2 | 57,872.4 | 68,266.0 | | |
| Volatile fatty acids | | | | | | | | | |
| 18.55 | 2-Methylpropanoic acid | 432.1 ± 20.2 ^b | 225.5 ± 3.8 ^{ab} | 1067.5 ± 181.5 ^c | 41.0 ± 7.7 ^a | 407.8 ± 114.9 ^b | 145.4 ± 19.5 ^{ab} | | |
| 20.37 | Butanoic acid | 47.8 ± 1.0 ^{ab} | 25.2 ± 2.2 ^a | 65.3 ± 1.9 ^b | 20.3 ± 0.3 ^a | 39.2 ± 17.2 ^{ab} | 63.4 ± 8.1 ^b | | |
| 28.08 | Hexanoic acid | 969.6 ± 13.6 | 660.5 ± 119.6 | 1359.1 ± 200.2 | 893.4 ± 1.1 | 769.4 ± 136.2 | 1306.8 ± 36.4 | | |
| 35.17 | Octanoic acid | 1592.6 ± 33.9 | 1096.5 ± 103.6 | 1452.9 ± 202.0 | 1807.9 ± 83.0 | 1327.3 ± 92.5 | 1974.4 ± 239.3 | | |
| 41.69 | Decanoic acid | 135.3 ± 5.9 | 81.3 ± 9.1 | 108.1 ± 12.0 | 87.4 ± 11.8 | 137.5 ± 13.2 | 133.3 ± 5.5 | | |
| 49.22 | Phenylacetic acid | 269.0 ± 4.2 | 207.0 ± 17.0 | 530.4 ± 18.3 | 200.2 ± 13.5 | 308.4 ± 60.1 | 350.2 ± 61.9 | | |
| | Subtotal | 3446.4 | 2296.0 | 4583.3 | 3050.2 | 2989.6 | 3973.5 | | |
| Lactones and terpenes | | | | | | | | | |
| 34.02 | Pantolactone | 37.2 ± 2.1 | 21.5 ± 2.5 | 92.0 ± 6.8 | 17.5 ± 0.5 | 32.2 ± 15.0 | 47.7 ± 4.1 | | |
| 13.52 | trans-Furan linalool oxide | 666.1 ± 10.3 | 509.4 ± 50.6 | 1025.0 ± 71.9 | 701.8 ± 8.3 | 676.7 ± 89.0 | 839.3 ± 10.2 | | |

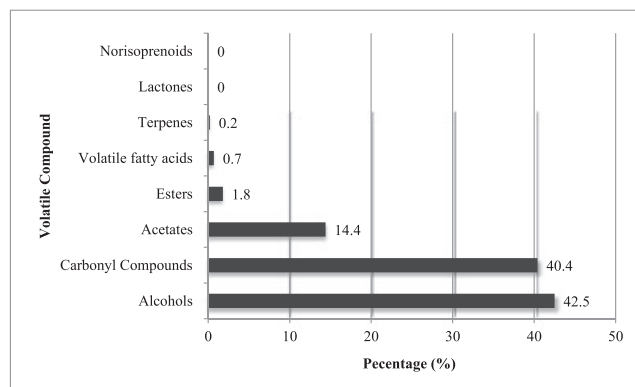
(Continues)

Table 3. (Continued)

| Retention time | Identified volatile compounds ($\mu\text{g/L}$) | Mead (one fining agent) | | | | Mead (two fining agents) | | | |
|----------------|---|-------------------------|--|-------------------------|--|--------------------------|--|----------------------------------|------------------|
| | | B | | T | | S | | TS | |
| | | $\square \pm \text{SD}$ | | $\square \pm \text{SD}$ | | $\square \pm \text{SD}$ | | $\square \pm \text{SD}$ | |
| 14.54 | cis-Furan linalool oxide | 263.3 \pm 0.8 | | 229.7 \pm 26.4 | | 434.3 \pm 65.8 | | 287.4 \pm 33.2 | |
| 19.82 | Ho-trienol | 158.6 \pm 0.6 | | 151.6 \pm 11.8 | | 256.4 \pm 19.5 | | 161.7 \pm 17.8 | |
| 22.81 | α -Terpineol | 18.8 \pm 0.7 | | 12.6 \pm 2.1 | | 59.9 \pm 5.7 | | 21.9 \pm 2.4 | |
| | Subtotal | 1106.8 | | 903.3 | | 1775.6 | | 1147.7 | |
| Norisoprenoids | | | | | | | | | |
| 22.51 | 4-Oxo-isophorone | 26.2 \pm 1.1 | | 19.3 \pm 2.5 | | 55.3 \pm 6.1 | | 26.5 \pm 5.1 | |
| | Total | 484,568.8 | | 419,867.0 | | 691,165.2 | | 471,575.6 | |
| | | | | | | | | 34.5 \pm 6.4 | 473,612.1 |

* Major volatile compounds (quantified by GC-FID). Means with different superscripts letters (^{a-d}), are significantly different ($p < 0.05$). Lack of a superscript letter indicates no significant difference ($p > 0.05$).

B, Bentonite, gelatine and egg albumin; S, silica; T, tannins; G, gelatine; BS, bentonite, gelatine and egg albumin + silica; TS, tannins + silica; SG, silica + gelatine.

**Figure 1.** Main groups of volatile compounds found on mead samples.

samples, ethyl acetate was the predominant compound with a concentration ranging from 52.3 to 114.4 mg/L, followed by isoamyl acetate and 2-phenylethyl acetate. Our results are in agreement with those reported by Oliveira *et al.* (32) in wines, who also found higher concentrations of ethyl acetate. According to Etievant *et al.* (33) concentrations of ethyl acetate >200.0 mg/L may have negative effects on wine aromas. The results of this study are lower than this.

In our study, the highest concentration of ethyl acetate was attained for sample S, which was considered the second most appreciated by the consumers perhaps owing to its fruity aroma. Also, these results are in agreement with the literature (3,34) showing that silica gel has an advantage over bentonite because it does not cause significant alterations in the organoleptic characteristics of the wine. In sample B, the concentration of ethyl acetate was approximately half that observed in sample S. Also the isoamyl acetate and 2-phenylethyl acetate concentrations were decreased by the action of bentonite + gelatine + egg albumin. Other groups that decreased their concentrations owing to the action of B (bentonite + gelatine + egg albumin) were lactones and terpenes but with silica the concentrations of these groups increased. Significant differences ($p < 0.05$) were only found for ethyl acetate.

Esters

Esters (1.8%) represented by monoethyl succinate with concentrations ranging from 0.4 to 15.4 mg/L, followed by diethyl succinate, ethyl lactate, ethyl hexanoate, diethyl malate, ethyl butyrate, ethyl octanoate and ethyl-3-hydroxy-butanoate. These results are in agreement with Patel and Shibamoto (25), who found monoethyl succinate in the highest concentrations in Petite Sirah grapes fermented with seven different *Saccharomyces* yeast strains. Also Selli *et al.* (35) found higher levels of monoethyl succinate in red wine from cv. Kalecik Karasi grown in central Anatolia. Significant differences ($p < 0.05$) were observed in monoethyl succinate, ethyl octanoate and ethyl-3-hydroxy-butanoate.

Volatile fatty acids

Volatile fatty acids (0.7%) represented by octanoic acid that presented the highest concentrations (1.1–2 mg/L), followed by hexanoic acid, 2-methylpropanoic acid, phenylacetic acid, decanoic acid and butanoic acid. Similar results were observed

by Vilanova *et al.* (17), who reported higher concentrations of octanoic acid in Gewürztraminer white wines. Significant differences ($p < 0.05$) were observed in butanoic acid and 2-methylpropanoic acid.

Terpenes, lactones and norisoprenoids

For the remaining groups, terpenes were more representative with four compounds: *trans*-furan linalool oxide followed by *cis*-linalool oxide furan, *ho*-trienol and α -terpineol. Lactones and norisoprenoids were represented by pantolactone and 4-oxo-isophorone. The concentrations of these compounds did not show significant differences ($p > 0.05$) in the mead samples under study. These results are in agreement with Mateo and Jiménez (36), who reported that terpenes are not influenced by the metabolism of yeast during fermentation.

In general (see Table 3), it was found that the tannins caused a significant decrease in the concentrations of volatile compounds. The groups of compounds most affected by tannins were alcohols, volatile fatty acids and carbonyl compounds. Another fining agent that affected considerably the volatile composition was the combined (B) fining agents with bentonite. Bentonite has been reported to be responsible for loss of wine aroma, a negative sensorial perception that is a serious problem in oenology (3). In our study the bentonite, despite reducing the volatile compounds, had no negative effect on the sensory characteristics of mead.

Also, these results suggested that the combination of two fining agents may decrease concentrations of the majority of studied volatile compounds, probably affecting negatively the aroma of these beverages. From the total of 36 identified volatile compounds, 12 (33.3%) were significantly affected by the fining used ($p < 0.05$), suggesting that the fining can influence the quality of mead, which corroborates with previous observations (3).

Volatile compounds with higher impact on mead

In Table 4 are listed the 11 volatile compounds (OAVs > 1) identified, as well as their odour thresholds and aroma descriptors. According to Oliveira *et al.* (19), compounds with OAVs > 1 make an active contribution to the aroma of wines. However, in some cases, even though a compound has an OAV > 1 , this does not mean that the compound will be perceived in a wine. On the other hand, it has been reported that compounds with OAVs < 1 may contribute to wine aroma through an additive effect of compounds with a similar structure or odour (37).

From all compounds that exhibited OAVs > 1 , acetaldehyde, ethyl butyrate, ethyl acetate, isoamyl acetate, ethyl hexanoate, 3-methyl-1-butanol, ethyl octanoate, *ho*-trienol, 2-phenylethanol, hexanoic acid and octanoic acid showed concentrations above their odour threshold; therefore, they represent those compounds with a major impact on the aroma of these beverages. However, Rocha *et al.* (38) in their studies reported the relevance in the overall aroma of substances at concentrations of $\geq 20\%$ of their threshold levels (OAVs > 0.2). Therefore, when considering the concentrations OAVs > 0.2 , 2-methyl-1-propanol, 2-methyl-1-butanol and phenylacetic acid must be considered as volatile compounds, which may also play an important role in the mead under study.

Esters

The group of esters (27.3%) was represented by ethyl butyrate, ethyl octanoate and ethyl hexanoate. Ethyl octanoate was the compound that exhibit the highest OAVs in the mead samples under study. These results are in agreement with Pereira *et al.* (12), who found ethyl octanoate to be the most powerful odourant in mead. The results are similar to those recently reported by Pereira *et al.* (21). These authors reported the presence of ethyl octanoate, isoamyl acetate and ethyl hexanoate as important odourants in

Table 4. Odour threshold, odour activity values (OAVs > 1) and aroma descriptor of volatile compounds with mayor impact on the mead treated with different finings

| Volatile compounds | Odour threshold (µg/L) | OAVs >1 | | | | | | Aroma descriptor |
|--------------------|------------------------|-------------------------|------|------|--------------------------|------|------|---|
| | | Mead (one fining agent) | | | Mead (two fining agents) | | | |
| | | B | T | S | TS | SG | BS | |
| Acetaldehyde | 10,000 (22) | 14.7 | 16.1 | 30.9 | 26.1 | 16.2 | 18.5 | Pleasant, fruity (20), overripe bruised apples, sherry-like, stewed apple (29), pungent and ripe apple (22) |
| Ethyl butyrate | 20 (17) | 2.2 | 1.2 | 2.9 | 2.2 | 2.4 | 4.0 | Papaya, butter, sweetish (17), fruity, sweet (40) |
| Ethyl acetate | 7500 (22) | 7.0 | 8.1 | 15.3 | 12.1 | 7.7 | 9.1 | Fruit, solvent (50), solvent, fruity (45), pineapple, balsamic (46) |
| Isoamyl acetate | 30 (17) | 2.0 | 2.1 | 9.7 | 3.4 | 1.7 | 2.7 | Banana (40,43) |
| Ethyl hexanoate | 14 (17) | 17.2 | 8.6 | 10.4 | 16.8 | 9.9 | 21.9 | Apple, fruity, sweetish (17), green apple, anise (40,51), strawberry (52) |
| 3-Methyl-1-butanol | 30,000 (17) | 4.6 | 3.4 | 3.9 | 3.1 | 4.0 | 3.6 | Cheese (44), warm, herbaceous, slightly fruity, nut-like, penetrating, acrid at high levels (29) |
| Ethyl octanoate | 5 (17) | 21.4 | 11.6 | 12.0 | 29.4 | 18.4 | 42.0 | Apple, sweetish (17), burned, beer (40) |
| Ho-trienol | 110 (48) | 1.4 | 1.4 | 2.3 | 1.5 | 1.5 | 1.9 | Linden (48) |
| 2-Phenylethanol | 10,000 (17) | 5.0 | 3.1 | 3.3 | 2.0 | 4.2 | 3.4 | Rose, sweetish (17), flowery, pollen, perfume (40) |
| Hexanoic acid | 420 (17) | 2.3 | 1.6 | 3.2 | 2.1 | 1.8 | 3.1 | Geranium, vegetable (17) |
| Octanoic acid | 500 (17) | 3.2 | 2.2 | 2.9 | 2.3 | 2.7 | 3.9 | Sweat, cheese (17) |

meads. Indeed these results are similar to those reported by several investigators in similar beverages, who demonstrated that ethyl octanoate plays an important role in wine aroma (17,23,39–42).

In general, ethyl octanoate and ethyl hexanoate have been reported as conferring good characteristics and pleasant aromatic properties even in low concentrations (27), conferring apple, burned and beer flavours, fruitiness, sweetness and freshness to wines (17,22,23,40). Another compound that has been pointed out to be responsible for the fruity and strawberry flavours of wine is ethyl butyrate (18,43,44).

Alcohols

This group represents 18.2% of the total volatile compounds with OAVs >1 and is represented by 3-methyl-1-butanol. This compound is characterized by assigning characteristics such as warm, herbaceous, slightly fruity, nut-like, penetrating and acrid after taste, with, at high levels, cheese and nail polish flavours (22,29,44). The second most abundant compound was 2-phenylethanol, which confers aromatic notes such as flowery, mostly roses and pollen, as well as sweet and perfumed flavours, to the beverage (17,22,23,40,44,45).

Acetates

The acetates, with mean percentages of 18.2%, were represented by ethyl acetate and isoamyl acetate. They have been reported to be one of the compounds that positively affects the organoleptic characteristics of wine, contributing to its improved aroma (46). According to Meilgaard *et al.* (45) and Peinado *et al.* (47), the presence of ethyl acetate in the wine gives a fruity, pineapple, solvent and balsamic after taste. Another acetate that expressed great impact on the mead's aroma in this study was isoamyl acetate, which has been reported to give fruity, banana and apple tastes (43).

Volatile fatty acids

The volatile fatty acids (18.2%) were predominantly represented by octanoic acid and hexanoic acid, compounds that have been reported as conferring geranium, vegetable, sweat and cheese aromas to wines (17,18).

Carbonyl compounds

Of the carbonyl compounds (9.1%), acetaldehyde was a unique compound with great impact on the aromatic profile of the mead under study. This compound exhibited the highest OAVs in the samples treated using a single fining agents.

Terpenes

Terpenes, also at 9.1%, were represented by α -terpinol. According to Clarke and Bakker (48), this compound is responsible for the linden aroma. It has also been reported that terpenes may play an important role in the taste of a particular wine, specifically in general flavour perception, owing to their characteristic aroma (28,49).

In Table 4 are presented 11 of the 36 quantified volatile compounds, whose concentrations were above the corresponding thresholds and may play an important contribution to mead's aroma and flavour, because their concentrations were above their corresponding thresholds. As mentioned above, ethyl octanoate is the volatile compound with the greatest impact on the quality of

the studied mead samples. Moreover, it can be seen that, out of the total of 11 (30.6%) compounds that exhibited OAVs >1, four were majority volatile compounds and the remaining seven were minority compounds.

Sensory analysis

In this work, the influence of some fining agents on the mead characteristics was tested using principal component analysis, which is one of the most commonly used multivariate techniques for wine analysis (17). In Fig. 2(a), the results of principal component analysis show that mead samples are clearly separated by two components (F1 and F2), which explain 86.8% of the total variability. The first (F1) component represents 66.9% and is highly correlated with aroma, flavour, sweetness and the general assessment. The second (F2), representing 19.9% of the total variability, is highly correlated with AS. Furthermore, it can be seen that the analysed meads were well separated according to fining agents used other than T and BS samples. It can also be seen that most sensory attributes evaluated are highly correlated with the first dimension, so 66.9% of the variability is concentrated in this

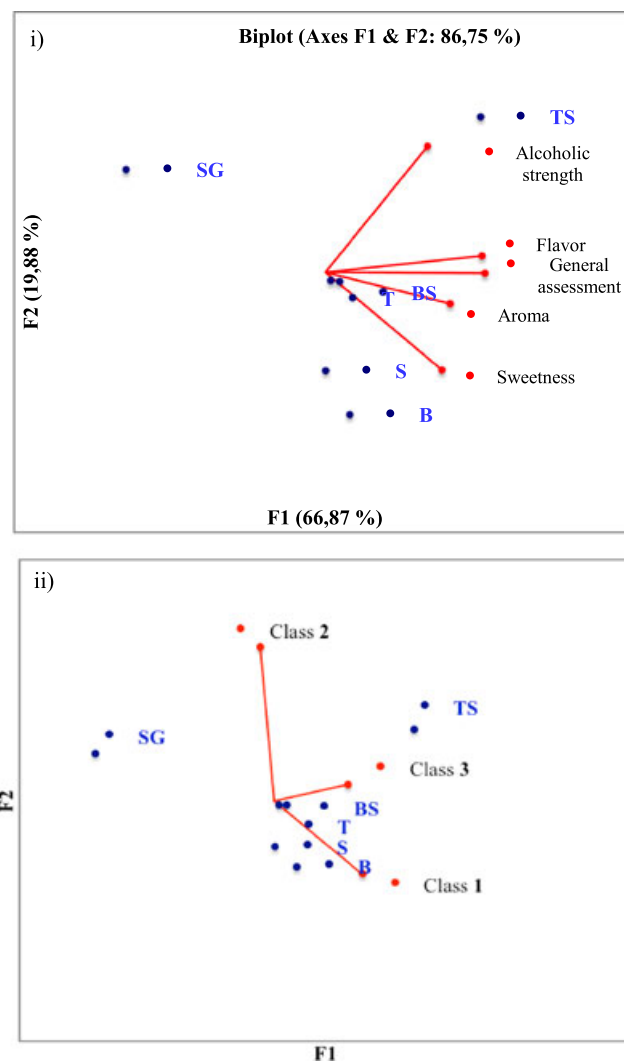


Figure 2. Results from the sensory analysis: (a) correlation between sensory parameters and mead samples; and (b) preference map. [Colour figure can be viewed at wileyonlinelibrary.com]

dimension. It is important to highlight that the data on the evaluated attributes show consumer preferences and not the intensity of characteristics. The obtained map, allows representation of 86.8% of the total variability, allowing us to prove that the products were preferred by consumers quite differently. The sensory parameters and mead samples correlate highly and positively with the same factor/F1 axis (66.9%) except for the SG sample, which is peculiar. This indicates that the consumers were able to perfectly distinguish the influence of fining in the analysed mead samples. The meads that received higher grades by the consumers were samples B and S, for sweetness, aroma evaluation and general assessment.

Figure 2(b) shows that class 1, comprising by 25 consumers, preferred meads B and S, characterized by their higher scores in sweetness, aroma and general assessment. Even though class 1 did not like SG mead characterized by the taste resulting from the mixture of two fining agents (silica + gelatine), class 2 comprising nine consumers liked this mead. The third class, also comprising 9 consumers, preferred mead that resulted from a mixture of two finings, tannins and silica, characterized by their higher preference in AS, flavour and general assessment.

Regarding the consumers' evaluation of the final product, the highest percentage (80%) was assigned for samples B (bentonite + gelatine + egg albumin) and S (silica), therefore these were preferred by the consumers. The remaining samples (SG, TS and T) had the same percentage (60%) and sample BS (bentonite + gelatine + egg albumin + silica) presented the lowest percentage (40%). The most interesting of these results was the fact that consumers liked mead fined by silica and combined fining agents (bentonite + gelatine + egg albumin) separately; however the combination of both was less pleasant for them.

Inter-relations between sensory analysis and OAVs

Comparing the results of sensory analysis and OAVs, it was verified that the meads B and S, which received the best score, presented lower OAVs on ethyl octanoate, ethyl butyrate, ethyl hexanoate and octanoic acid compared with the sample BS, which proved to be the least appreciated of all analysed samples. However, for the remaining volatile compounds – acetaldehyde, ethyl acetate, isoamyl acetate, 3-methyl-1-butanol, *ho*-trienol, 2-phenylethanol and hexanoic acid – the OAVs of these compounds were in general way slightly higher than those observed in sample BS. Therefore, the best scored samples exhibited more volatile compounds (7) with higher level of OAVs than the sample BS, which resulted in a worse evaluation by the group of consumers.

Conclusions

According to the results reported here, the lower and medium fining agents concentrations proved to be ineffective for mead clarification. Therefore, the highest concentrations presented the best results. It may be concluded that the concentration and type of fining agents used significantly influence the composition of the final mead.

In general, a trend to decrease the concentrations of volatile compounds with the use of two clarifiers was verified. Indeed, it was only for four compounds (ethyl octanoate, octanoic acid, decanoic acid and *trans*-furan linalool oxide) that there was observed a tendency for increasing concentration with the use of combined fining agents.

Only the mead B, treated by combined fining agents (bentonite + gelatine + egg albumin), presented superior alcohol concentrations. Silica (S) was the fining agents that exhibited higher concentrations of volatile compounds, particularly regarding esters, volatile fatty acids, lactones, terpenes and norisoprenoids. This suggests that these may be the reason why this sample was one of the preferred by consumers.

Tannins were the fining agent that significantly decreased the volatile compounds in all samples under study. In general, we found that the reduction of volatile compounds in mead is not related to the use of just one or two finings but, instead, depends on the type of fining used. Looking at the results, the samples S and BS treated with silica were the samples that presented a higher level of OAVs in all samples under study.

These findings may be useful for the practical selection of fining agents for use in mead. In any case, future studies should be conducted with other fining agents in different concentrations in order to determine which can provide the optimum qualities for consumers of such beverages.

Conflict of interest

The authors declare that there are no conflicts of interest. They are indebted for the careful and constructive criticisms of the reviewers.

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